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Notes on rust fungi in China 2. Two species of *Coleosporium* on *Compositae*

Jing-Xin Ji [1], Qi Wang [1], Zhuang Li [2], Yu Li [1] & Makoto Kakishima [1, 3*]

[1] *Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, Jilin 130118 China*

[2] *College of Plant Pathology, Shandong Agricultural University, Tai'an 271000 China*

[3] *University of Tsukuba, Tsukuba, Ibaraki 305-8572 Japan*

* Correspondence to: *kakishima.makoto.ga@u.tsukuba.ac.jp*

Abstract—During investigations of rust fungi in northeast China, specimens of *Coleosporium* on various *Compositae* were collected. Based on comparative morphology and molecular phylogenetic analysis specimens on *Hieracium, Parasenecio*, and *Senecio* were identified as *C. neocacaliae*, and those on *Ligularia, Saussurea*, and *Synurus* as *C. saussureae*. *Hieracium umbellatum* is a new host for *C. neocacaliae*, and *Saussurea odontolepis* and *S. pulchella* are new hosts for *C. saussureae*. *Coleosporium synuricola* is treated as a synonym of *C. saussureae*.

Key words—*Pucciniomycetes*, rust flora, taxonomy, *Uredinales*

Introduction

The rust genus *Coleosporium* is characterized by 1-celled teliospores that are produced in a 1-layered crust under host epidermal cells (Cummins & Hiratsuka 2003). The teliospores germinate without dormancy and produce 4-celled internal basidia. About 240 species have been described (data from MycoBank) and most of the species have a heteroecious life cycle producing spermogonia and aecia on needles of pines and uredinia and telia on various angiosperms (Kaneko 1981). In China about 45 species have been reported (Miura 1928, Ito 1938, Tai 1979, Cao & Li 1999, You et al. 2010), but their taxonomy and host plants are not well circumscribed and life cycles are still not investigated.

During investigations of rust fungi in Jilin Province, northeast China, we collected rust specimens on species of *Compositae* (*Hieracium, Ligularia*,

Parasenecio, Saussurea, Senecio, Synurus). These rust fungi have been referred to the genus *Coleosporium*, but species identifications are sometimes confused because of morphological similarities among species reported on these host plants. After morphological examinations and phylogenetic analyses, specimens on *Hieracium, Parasenecio*, and *Senecio* were identified as *C. neocacaliae* and those on *Ligularia, Synurus*, and *Saussurea* were identified as *C. saussureae*. *Hieracium umbellatum* L. was recorded as a new host plant for *C. neocacaliae* and *Saussurea odontolepis* (Herder) Sch.-Bip. ex Herder and *S. pulchella* (Fisch.) Fisch. were recorded as new hosts for *C. saussureae*. The identity of *Coleosporium synuricola* Y. Xue & L.P. Shao, described on *Synurus deltoides* (Aiton) Nakai, was clarified, and it was shown to be identical to *C. saussureae*.

Materials & methods

Specimens

Specimens on *Compositae* were collected mostly in the vicinity of Changbai Mountain, Jilin Province, China, from 2013 to 2015. Some specimens deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU) were also examined. Their host plants were *Hieracium umbellatum*, *Ligularia fischeri* (Ledeb.) Turcz., *Parasenecio hastatus* (L.) H. Koyama (≡ *Cacalia hastata* L.), *Saussurea odontolepis*, *S. pulchella*, *S. umbrosa* Kom., *Senecio nemorensis* L., and *Synurus deltoides*. The specimens used for morphological observations and molecular analysis were deposited in HMJAU.

Morphological observations

Light microscopy (LM) was used to examine morphological characters including the size and shape of sori and spores. Spores or thin-sections of sori from specimens were mounted in a drop of lactophenol solution on glass slides for LM. Approximately 20 spores from each specimen were randomly chosen and the length, width, and wall thickness of spores were measured using Leica LAS X software attached to a Leica DM2000 microscope (Leica, Germany).

The surface features of spores were examined by scanning electron microscopy (SEM). For SEM, samples obtained from dry specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium using a Hitachi MC1000 Ion Sputter Coater and examined with a Hitachi SU8010 SEM operated at 5–7 kV.

DNA extraction and sequencing

The total genomic DNA was extracted from about 200 spores obtained from a single uredinium or telium. Spores were crushed between two sterilized glass slides and suspended in 30 µl extraction buffer [10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% sodium dodecyl sulfate (SDS), 0.01% Proteinase K], and the suspensions were incubated at 37°C for 1 hour and 95°C for 10 min, followed by a 4°C soak (Suyama et al. 1996, Virtudazo et al. 2001). From the crude extract, 5–7 µl

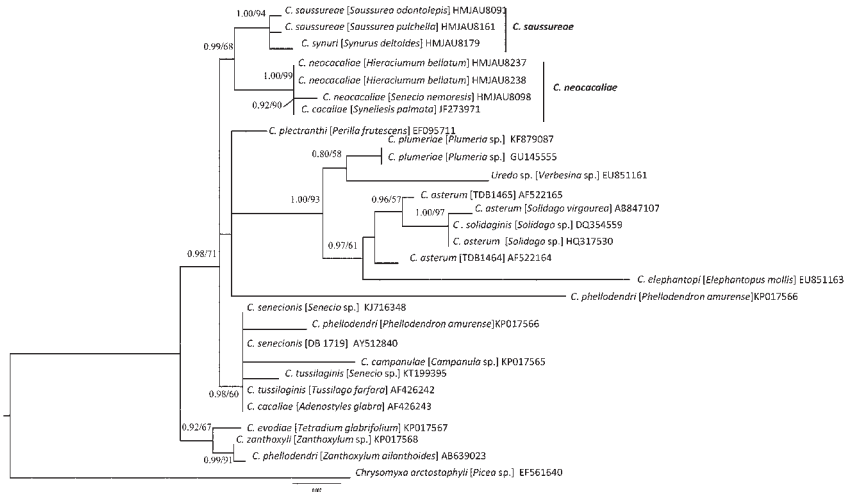

Figure 1. Phylogenetic tree of *Coleosporium*. Bayesian 0.5 majority-rule consensus tree based on the 28S RNA gene. *Chrysomyxa arctostaphyli* was used as outgroup. Values on the branches indicate maximum likelihood bootstrap values / Bayesian posterior probabilities. Bootstrap values <75% and Bayesian posterior probabilities <0.75 are not shown on the branches.

samples were used directly for each polymerase chain reaction (PCR). The rDNA-28S region was amplified using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell 1993). The PCR amplifications were performed in 50 µl of mixture containing 5 µl of template DNA, 200 µµ of each primer, 25 µl of Premix Taq™ (TaKaRa Taq™ Version 2.0 plus dye) (TaKaRa, Tokyo, Japan), and 18 µl of ddH_2O. Cycling conditions for amplification consisted of 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were separated on 1% agarose gels containing Nucleic Acid Stain (Beijing Dinggou Changsheng Biotechnology Co. Ltd.) and purified using the TaKaRa MiniBEST Agarose Gel DNA Exaction Kit Ver.4.0. Purified PCR products were cloned in pEASY® -T1 Cloning Vector (Transgene Biotech, Beijing, China) and then transferred into Trans1-T1 phage, resistant chemically competent cell according to the manufacturer's instructions. The positive clones were sequenced by Sangon Biotech Co., Shanghai, China. All data sequenced in this experiment were deposited at GenBank (KX344988– 344993)

Phylogenetic analysis

The sequence data were manually aligned using BioEdit ver. 7.0.9 (Hall 1999). To make molecular comparison, 23 sequences were retrieved from GenBank based on host species and genus (FIG. 1). Multiple alignments were performed using MEGA6. The final dataset contained sequences from 29 specimens with a length of 610 bp, which included 120 parsimony-informative characters.

Phylogenetic trees were constructed with a sequence of *Chrysomyxa arctostaphyli* Dietel as the outgroup. Topologies were constructed based on maximum likelihood (ML) analyses using RaxmalGUI1.5b1. Bayesian Markov chain Monte Carlo (MCMC) analyses were performed using MrBayes ver. 3.1.2 (Huelsenbeck & Ronquist 2001). In ML and Bayesian analyses, the best-fit substitution models were estimated using Modeltest ver. 3.7 (Posada & Crandall 1998), and K81uf+I+G was selected as the best evolutionary model.

Results & discussion

Specimens on *Hieracium, Parasenecio*, and *Senecio*

Specimens on *H. umbellatum, P. hastatus*, and *S. nemorensis* were morphologically similar and their uredinial and telial characteristics were identical to the descriptions of *C. neocacaliae* (Kaneko 1981, Azbukina 2005). Molecular phylogenetic analysis of the rDNA 28S regions based on ML and Bayesian inference placed the specimens on *H. umbellatum* (HMJAU8237 and HMJAU8238) and *S. nemorensis* (HMJAU8098) in the same lineage (Fig. 1). GenBank data of a rust from Korea recorded as *C. cacaliae* (DC.) G.H. Otth on *Syneilesis palmata* (Thunb.) Maxim. [= *Cacalia thunbergii* Nakai] (JF273971)

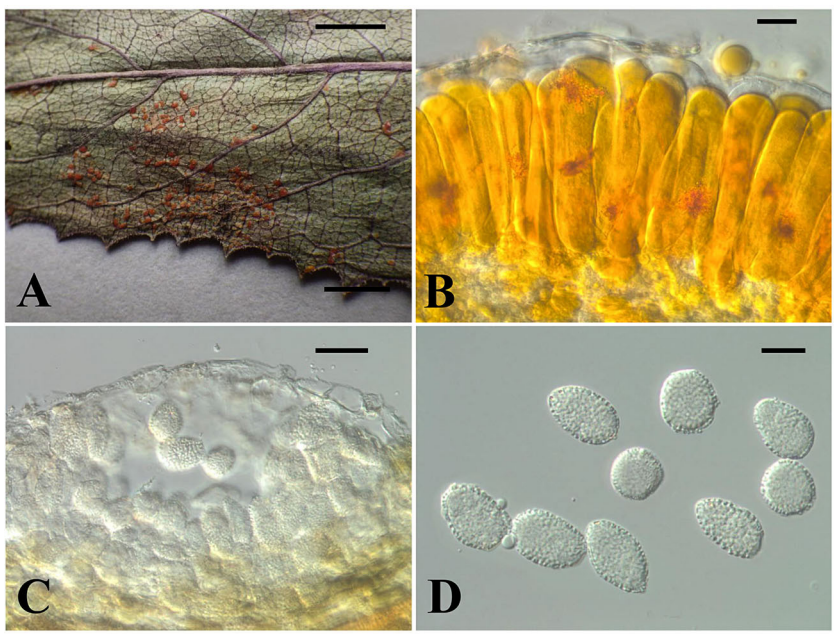

Figure 2. ***Coleosporium neocacaliae*** on *Hieracium umbellatum*. A. Telia on lower leaf surface. B. A vertical section of a telium. C. Vertical section of uredinium. D. Urediniospores. Scale bars: A = 1 cm; B–D = 20 μm.

was also included in this lineage. However, this plant was reported as a host of *C. neocacaliae* (Kaneko 1981) and it is phylogenetically distinct from *C. cacaliae* from Europe (Fig. 1, AF426243). Based on these specimens a description of *C. neocacaliae* is provided.

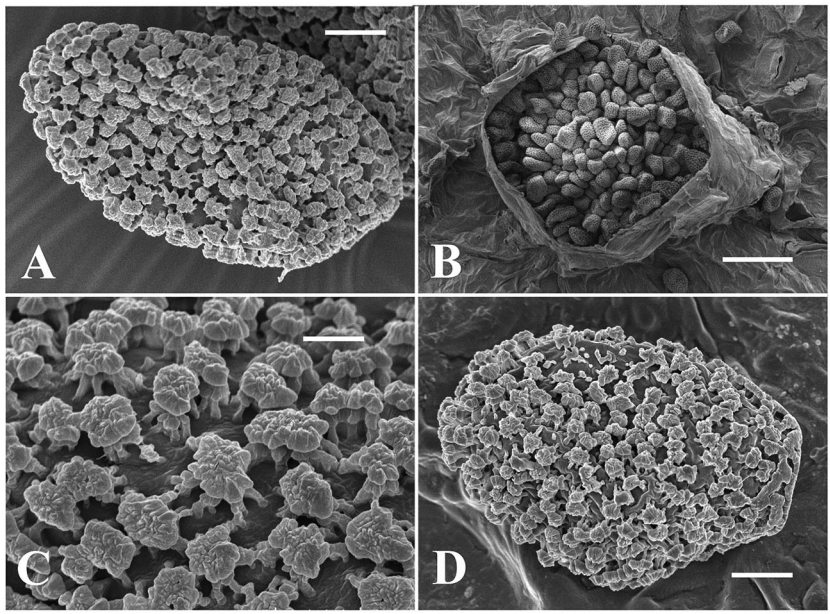

Figure 3. ***Coleosporium neocacaliae***. A. Urediniospore on *Hieracium umbellatum*. B. Uredinium on *H. umbellatum*. C. Surface of urediniospore on *H. umbellatum*. D. Urediniospore on *Senecio nemorensis*. Scale bars: A, D = 5 μm; B = 50 μm; C = 2 μm.

Coleosporium neocacaliae Saho, Trans. Mycol. Soc. Japan 7: 59, 1966. Figs 2, 3

Uredinia hypophyllous, scattered, orange-yellow, pulverulent, globoid; urediniospores ellipsoid, broad-ellipsoid, 16.0–34.5 × 13.0–21.5 μm (av. 24.5 × 16.5 μm), walls uniformly thick 0.3–1.5 μm (av. 1.0 μm), verrucose with a smooth reticulum-like spot. Telia hypophyllous, scattered, rounded, orange-red; one-celled teliospores cylindrical, clavate or oblong-ellipsoid, 44–82 × 16–27 μm excluding gelatinous apical layer, four-celled internal basidia 60–107 × 21–34 μm, longitudinally or obliquely septate, mature teliospores or basidia arranged in a single layer, gelatinous apical layer 8–10 μm thick, wall thin, hyaline.

Specimens examined — **China, Jilin Province:**

on *Hieracium umbellatum*: Changbai Mt., stage II, 29 July 2015 (HMJAU8237; GenBank KX344992); stage III, 23 September 2015 (HMJAU8238; GenBank KX344993);

on *Parasenecio hastatus*: Lushuihe, Baishan, stage II, 21 July 2002 (HMJAU8077, 8078, 8079); Changbai Mt., 29 July 2015, (HMJAU8263);

on *Senecio nemorensis*: Changbai Mt., stage 11, 23 July 2002 (HMJAU8098; GenBank KX344991).

Coleosporium neocacaliae has been reported only on species of *Cacalia* and *Senecio* from the Russian Far East, China, Korea, and Japan (Tai 1979, Kaneko 1981, Hiratsuka et al. 1992, Azbukina 2005), which makes *H. umbellatum* a new host plant for *C. neocacaliae*. This species differs in telial morphology from *C. cacaliae* and *C. parvisporum* S. Kaneko reported on species of *Cacalia* and *Senecio* (Kaneko 1981). The 4-celled basidia of *C. parvisporum* are smaller than those of *C. neocacaliae*. The 4-celled basidia of *C. cacaliae* have sterile cells at their base, but those of *C. neocacaliae* have no sterile cell. Our molecular analysis also confirms a phylogenetic difference between *C. neocacaliae* and *C. cacaliae* (Fig. 1).

Specimens on *Ligularia, Saussurea*, and *Synurus*

Specimens on *Ligularia fischeri*, *Saussurea odontolepis*, *S. pulchella*, *S. umbrosa*, and *Synurus deltoides* were morphologically similar and uredinial and/or telial characteristics were identical with the descriptions of *C. saussureae* (Kaneko 1981, Azbukina 2005). Our DNA sequence analyses also supported specimens on *Saussurea pulchella* (HMJAU8161), *S. umbrosa* (HMJAU8091) and *Synurus deltoides* (HMJAU8179) in the same linage (Fig. 1). Therefore, these specimens were identified as *C. saussureae*. Rust on *Synurus deltoides* was reported as *C. synuricola* (Xue & Shao 1995, Azbukina 2005). However, we observed no morphological difference between specimens on *Ligularia* and *Saussurea* and so consider *C. synuricola* a synonym of *C. saussureae*. This synonymy is also supported by our molecular analysis. Based on these specimens a description is provided.

Coleosporium saussureae Thüm., Bull. Soc. Imp. Nat. Moscow 55: 212, 1880.

Figs 4, 5

= *Coleosporium. synuricola* Y. Xue & L.P. Shao, Acta Mycol. Sinica 14: 248, 1995.

Uredinia hypophyllous, scattered, rounded, pulverulent, orange-yellow; urediniospores ellipsoid, oblong-ellipsoid or subglobose 21.5–32.5 × 13.0–20.0 μm (av. 26.0 × 16.0 μm), verrucose, with a smooth reticulum-like spot, walls uniformly 0.5–2.0 μm (av. 1.0 μm) thick. Telia hypophyllous, scattered, rounded, orange-red; one-celled teliospores obovoid to cylindrical, 48.5–88.5 × 18.0–30.0 μm (av. 68.5 × 23.5 μm) excluding gelatinous apical layer, four-celled internal basidia 73.5–96.0 × 18.0–27.0 μm (av. 81.5 × 22.5 μm), longitudinally or obliquely septate, mature teliospores or basidia arranged in a single layer, adhering among teliospores, gelatinous apical layer 9.5–32.5 μm thick, wall

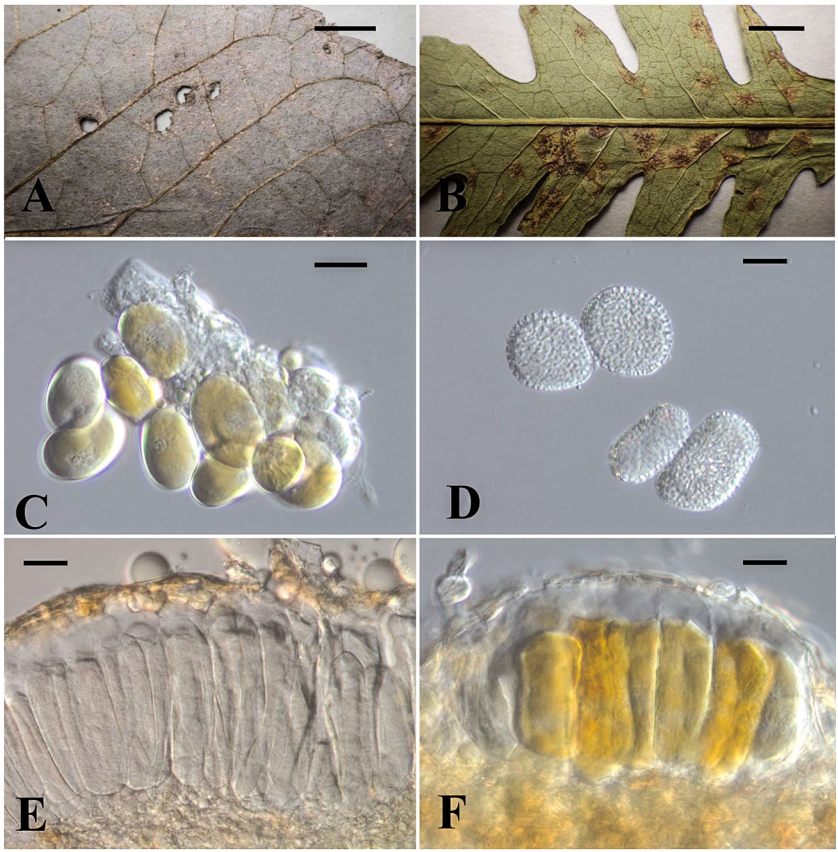

Figure 4. ***Coleosporium saussureae.*** A. Telia on lower leaf surface of *Synurus deltoides*. B. Telia on lower leaf surface of *Saussurea pulchella*. C. Basidiospores with smooth surface on *Synurus deltoides*. D. Urediniospores on *Saussurea umbrosa.* E. Teliospores on *Saussurea pulchella*. F. Teliospores on *Synurus deltoides*. Scale bars: A, B = 1 cm; C–F = 20 μm.

thin, hyaline; basidiospores ellipsoid, 23.0–29.5 × 15.0–18.5 μm (av. 25.5 × 16.5 μm), wall thin, hyaline.

Specimens examined — **CHINA, Jilin Province:**

on *Ligularia fischeri*: Yanbian, stages II, III, July 2015 (HMJAU8186);

on *Saussurea odontolepis*: Changbai Mt., stages II, III, 26 August 2014 (HMJAU8088); 27 August 2014 (HMJAU8089);

on *S. pulchella*: Changbai Mt., stage II, 29 July 2015 (HMJAU8161: GenBank KX344988); stages II, III, 15 September 2013 (HMJAU8143); stage II, III, 16 September 2013 (HMJAU8163); stage II, III, 24 August 2014 (HMJAU8086); stage III, 24 August 2014 (HMJAU8087, 8090); stage III, 23 September 2015 (HMJAU8266, 8267); Yanbian,

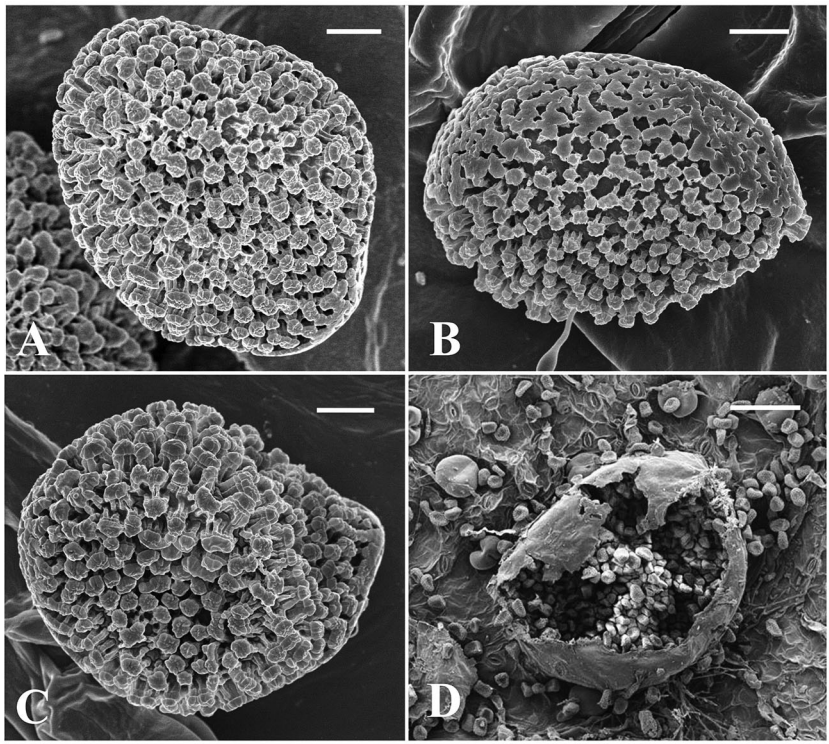

Figure 5. ***Coleosporium saussureae.*** A. Urediniospore on *Saussurea umbrosa*. B. Urediniospore on *S. odontolepis*. C. Urediniospore on *S. pulchella*. D. Uredinium on *S. pulchella*. Scale bars: A–C = 5 μm; D = 50 μm.

stage II, III, 30 July 2015 (HMJAU8165); stage II, III, 24 September 2015 (HMJAU8265); Yanji, stage III, 15 September 2013 (HMJAU8162, 8163, 8264); stage III, 17 September 2013 (HMJAU8146);

on *S. umbrosa*: Changchun, stage II, III, 11 September 2014 (HMJAU8091; GenBank KX344991);

on *Synurus deltoides*: Changbai Mt., stage III, 23 September 2015 (HMJAU8179; GenBank KX344989).

Coleosporium saussureae has been reported on *Ligularia* and *Saussurea* species and is known from the Russian Far East, China, Korea, Taiwan, and Japan (Kaneko 1981, Hiratsuka et al. 1992, Azbukina 2005). *Coleosporium pedunculatum* S. Kaneko was also described on species of *Saussurea*, but its teliospores have long sterile cells at their base, whereas *C. saussureae* has no sterile cell (Kaneko 1981). No sterile cells were observed in specimens on *Ligularia, Saussurea*, and *Synurus*. Therefore, we identified them as *C. saussureae.*

Ito (1938) and Tai (1979) listed *Saussurea odontolepis* and *S. pulchella* as host plants of *C. saussureae*. Later, Kaneko (1981) segregated *C. pedunculatum* from *C. saussureae* and treated them as host plants of *C. pedunculatum,* but specimens on these plants had no sterile cells on the teliospore bases and so were identified as *C. saussureae*. Therefore, these two plant species are new hosts for *C. saussureae* as revised by Kaneko (1981).

Geographical distribution of *C. saussureae* differs from that of *C. pedunculatum* due to the distributional differences of their aecial host species of *Pinus* (Kaneko 1981). *Coleosporium pedunculatum* is found mainly in warm regions, whereas *C. saussureae* occurs in alpine and mountainous regions, reflecting distribution of the aecial hosts. We collected specimens from Changbai Mt., an alpine (cold) region in China. Therefore, this identification of specimens is also supported by their distribution.

Coleosporium ligulariae Thüm. and *C. zhuangii* C.M. Tian & C.J You have been recorded on species of *Ligularia* from China (You et al. 2010). These species were reported to differ slightly in size and urediniospore surface structure from *C. saussureae*, but they are morphologically similar to each other. Comparative morphological studies of these species are needed to clarify their relationships.

Coleosporium synuricola [= *Uredo synuri* Azbukina] was described based on a specimen collected on *Synurus deltoides* in Heilongjiang Province, China, which is geographically close to Jilin Province (Kaneko 1981, Xue & Shao 1995, Azbukina 2005). This species is morphologically similar to *C. saussureae* as reported by Kaneko (1981) and Xue & Shao (1995). We confirmed morphological and phylogenetic identity between them based on the specimen on *S. deltoides*. Therefore, we treat *C. synuricola* as a synonym of *C. saussureae*.

Acknowledgments

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